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COMPARATIVE CYTOGENETIC STUDY IN FOUR ALOPIINAE DOOR SNAILS (GASTROPODA, CLAUSILIIDAE)

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ABSTRACT

A cytogenetic study was carried out by means of standard and NOR-FISH staining methods on the chromosomes of four Alopiinae door snails, two species of the Delimini tribe (Siciliaria paestana and Papillifera bidens) and two species of Medorini tribe (Leucostigma candidescens and Medora sp.). The two Delimini species and Medora sp. showed similar karyotypes, con-cerning the number of chromosomes, 2n = 62, and the first metacentric pair of chromosomes clearly bigger than the other pairs, but differed in the number of the telocentric pairs: none in P. bidens, four in S. paestana and, even, 21 in Medora sp. Leucostigma candidescens had 2n = 48, all biarmed chromosomes. Loci of NORs were on a single medium small chromo-some pair in all the four studied species. The obtained results evidence a relevant degree of intergeneric karyotypic diversity in Alopiinae, concerning both chromosome number and/or shape. The data allow us to make a hypothesis on the evolutionary chromosome trends for this door snail subfamily.
Key words: chromosome diversification, karyotype, NOR-FISH banding, Alopiinae, Clausiliidae.

INTRODUCTION

Alopiinae is a subfamily of the pulmonate
land snails of the family Clausiliidae, known
as door snails, because of the peculiar closing
apparatus, which derives from their sinistrorsal,
turriculate shell. The subfamily Alopiinae is particularly rich in species, comprising more than 360 living species distributed along the perimediterranean regions and in northern and southeastern Europe (Nordsieck, 2007; Uit de Weerd & Gittenberger, 2013). Nordsieck (2007), in his accurate and exhaustive systematic revi-sion of Clausiliidae, based on shell and genital characters, recognizes Alopiinae as a mono-phyletic assemblage, with the about 360 species subdivided in five tribes (Alopiini, Cochlodinini, Delimini, Medorini and Montenegrini). The mo-lecular phylogenetic analysis of Clausiliidae by Uit de Weerd & Gittenberger (2013) is in agree-ment with the morphological systematic revision of the family proposed by Nordsieck (2013a). Concerning Alopiinae, it retrieves monophyletic, as well as various tribes, including Delimini and Medorini (Nordsieck, 2013a). However,

many taxonomic, systematic and phylogenetic questions are not clearly defined. In this con-text, useful information can be gathered from other studies, such as chromosome analysis. In fact, the recognition of the main changes in karyotype composition of a given lineage may be helpful in understanding its evolutionary history. For example, the utility and efficiency of cytogenetic studies in exploring chiton taxa diversity has been recently shown, (Odierna et al., 2008; Petraccioli et al., 2012), highlighting the main evolutionary events of the karyotype diversification in two different taxa.
The present paper shows the results of a chromosomal study performed with standard and NOR-FISH staining methods on two spe-cies of Delimini, Siciliaria paestana (Philippi, 1836) and Papillifera bidens (Linnaeus, 1758) and two of Medorini. The two Delimini spe-cies belong to the Siciliaria group (Nordsieck, 2013b), considered monophyletic also in mo-
lecular phylogenetic studies (Uit de Weerd &
Gittenberger, 2013). The two Medorini species
analyzed were Leucostigma candidescens
(Rossmässler, 1835) and specimens of genus

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Medora, here designed as Medora sp., be-
cause the anatomical and molecular characters
of the samples collected were similar but not
perfectively matching with those of M. italiana
(Kuster, 1847), studied so far (Giusti, 1967,
1971; Giusti et al., 1986, Giusti & Manganelli, 1992: Reitano et al., 2007; Nordsieck, 2009, 2012; Colomba et al., 2012).
Alopiinae and Clausiliidae chromosomal data are scarce (for details, see Park, 2007) and mainly referred to the number of the chromo-somes. Only one karyotype of the Phaedusi-nae species, Euphaedusa fusaniana (2n = 58, all biarmed chromosomes) was described (Park, 2007). The data available for Alopiinae concern Herilla bosniensis and Cochlodina laminata, both with 2n = 48 (Thaler, 1963; Rainer, 1967), Delima itala and P. bidens, with tentatively 2n = 60 (Rainer, 1967). So far, no data were available for localization of NORs in Clausiliidae.

MATERIALS AND METHODS

We studied four specimens of Medora sp. and four specimens of S. paestana from Monti Alburni (Salerno), six of P. bidens from Aversa (Caserta) and five specimens of L. candide-scens from Cusano Mutri (Benevento). Chromosomes were prepared from gonads as described by Petraccioli et al. (2012). In brief, from each individual the gonads were removed and incubated for two hours in one ml of calf serum, inactivated at 56°C for 30 min in a solution containing 50 ul of colcemid at 10 ug/ml. The gonads were incubated for 30 min in hypotonic solution (KCI 0.075 M + sodium cit-rate 0.5%, 1:1) and fixed for 15 min in methanol: acetic acid, 3:1. Cell dissociations of gonads were made on a sieve and chromosomes were prepared for dropping on the slides 25 ul of the obtained cell suspensions.
Standard chromosome staining was per-formed by incubating chromosomes in DAPI at 0.5 ug/ml. After detection of metaphase plates with an epifluorescent microscope (Leica DM) equipped with a digital camera, the images were transformed in gray. For karyotyping, five metaphase plates of each species were used, which were also employed to get chromosome morphometric parameters, centromeric index (Cl) and relative length (RL) according to Levan et al. (1964).
NOR-FISH staining was performed as described by Petraccioli et al. (2012) using as a probe the biotinaled 18S rRNA gene

sequence units of the pectinid Adamussium colbecki. Slides were aged for one day at room temperature and then two hours at 60°C; after that, slides were incubated for 30 min in RNase at 100 pg/ml in Tris-HCI 10 mM at pH 6.5, dehydrated in ethanol 50, 70, 90 and 100% and air dried. Chromosomes and probe were denatured for 3 min at 72°C in hybridization mixture (10 ng/ml biotinylated 16 dUTP probe + 0.1 mg/ml shared E.coli DNA in 2 x SSC with 50% formamide). Hybridization was carried out at 40°C for 20 h. Washes were performed in 1 x SSC at 72°C for 5 min and at RT for 2 min. Probe detection was performed by monoclonal anti-biotin (Sigma cod. B7653) diluted 1:500 in РТВ (1 ml PTB = 5 ul of Tween 20% + 0.01 g of Dry milk in 1 ml of PBS 0.2 M). After one hour, slides were washed in 1 x PBS and incubated for 30 min in FITC conjugated anti-anti-biotin antibodies (Sigma cod. F9137) diluted 1:50 in PTB. Chromosomes were washed in 1 x PBS, counterstained with 5 ug/ml propidium iodide (PI) in 1 x PBS for 15 min at RT and then mounted with antifade (DABCO, Sigma). The hybridization signals were detected and recorded under an epifluorescent microscope (Leica DM) equipped with a digital camera.

RESULTS

Diakinetic meiotic bivalents and gonadic metaphase plates were successfully obtained from at least one individual of each studied species. Cytogenetic analysis showed that Medora sp., S. paestana and P. bidens had 31 bivalents and 62 chromosomes (Fig. 1). In the three karyotypes, only the first chromosome pair was clearly recognizable, shaped as a large metacentric, much bigger than the others (Fig. 1). Besides, the karyotype of three species differed in the number of biarmed and uniarmed elements. Medora sp. had a karyotype with ten biarmed pairs [8 metacentric (M), one sub-metacentric (SM) and one subtelocentric (ST)] and 21 pairs of telocentric (T) chromosomes (fundamental number, FN = 82) (Fig. 1, Table 1). In the karyotype of S. paestana, the number of biarmed chromosome pairs was 27 (25M, 2SM), and four pairs were telocentric, FN = 116) (Fig. 1, Table 1); instead all the chromosomes of P. bidens were biarmed (28M, 3SM), FN = 124 (Fig. 1, Table 1). Differently from the above three species, L. candidescens displayed a karyotype with 2n = 48 chromosomes, all bi-armed and progressively decreasing in length (22M, 2SM) FN = 96 (Fig. 1, Table 1).

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In metaphase plates of the four studied spe- Noteworthy, in the 25 scored diakinesis cies, NOR-FISH showed hybridization signals figures in each of the four studied specimens only on one small sized chromosome pair, while of S. paestana, some of them, namely, 2, 3, 3 their relative nuclei showed two signals (Fig. and 4 for specimen (in total 12%), presented a 2A) or one signal, due to NORs tendency tjoo in medium small sized achiasmatic bivalent with a in a one nuclear domain (Fig. 2B, C, D). gap on each of subterminal regions (Fig. 1).

D

FIG.1. Diakinetic meiotic plates (on the left) and karyotypes (on the right). A: L. candidescens; B: Medora sp.; C: P. bidens; D: S. paestana. The micrometer bar applies to all images. The arrows point to the gaps on the subteminal regions of an achiasmatic bivalent of S. paestana.

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FIG. 2. NOR-FISH on partial chromosome metaphase plates, with a relative nucleus in the insert. A: L. candidescens; В: Medora sp.; С: P bidens; D: $. paestana, in the inserts, the relative nucleus. The micrometer bar applies to all images.

Lampedusa lopadusae
Muticaria syracusana 4 2n=48 Leucostigma candidescens 24
Leucostigma candidescens + 3 № U Be © I a a OER RE I a te вия
Lu mu mm mem ee n
Agathylia lamellossa 1 2n=62 Medora sp.**
DA 38 Зв 35 375 à À 5 à 5 5 5 à à a à 6 à à

inchoatia haussknechti

Carinigera buresi
Albinaria puella
Isabellaria isabellina

Montenegrina dennisi

Alopia bielzii

Herilla bosniensis «————————— 2n=48 Неа bosniensis *
Cochlodina laminata «——————- 2n=48 Cochlodina laminata*
Cochlodina orthostoma
2n=60 Delima itala*
Delima hiltrudis ae nd Le ue” PN
Ee ee 1 2n=62 Siciliaria paestana
Charpentieria lamellata Pe GR Ree Rw ARR RARE A

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Charpentieria itala | 4 2n=62 Papillifera bidens
Macedonica pangaionica VOTE REIKI IT IT LK HE ахаха

FIG. 3. Phylogenetic tree of Alopiinae redrawn from the Clausiliidae tree by Uit de Weerd & Gittenberger (2013) with superimposed the so far known karyological data of Alopiinae. (* data by Thaler (1963) and Rainer (1967); ** see text, for the phylogenetic positions on the tree of $. paestana and Medora sp.).

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DISCUSSION

The karyotype of Medora sp. (2n =62; FN =
82) with a straight prevalence of telocentric ele-ments (21 pairs) is quite unusual among both gastropods and bivalves (Thiriot-Quiévreux, 2002, 2003), whose karyotypes, as a rule, include all or almost all biarmed (meta- and/

or submeta- centric) chromosomes. The other three investigated Alopiinae species do not deviate from this general rule, possessing a
karyotype with all biarmed chromosomes (P.
bidens and L. candidescens) or almost all
biarmed elements (S. paestana). In Alopiinae,
additional karyological data concern the chro-
mosome number of two species only, namely

TABLE 1. Morphometric parameters of chromosomes of the studied Alopiinae species. R.L. = Relative length; С.1. = Centromeric index; C.S = Chromosome shape (M= metacentric; SM = Submetacentric; ST = Subtelocentric and T = Telocentric).

L. candidescens Medora sp.

ChróNrao ri (Get! ER Gil

1 on! 44.1 M LaS 40.2
2 OrOry 43:2 М 4.8 43.2
3 49 456 M 4.5 45.1
4 4.9 40.2 M 4.2 40.3
5 48 42.8 M 4.1 0
6 4.8 46.6 M 4.1 0 if 45 44.3 M 3.9 329 8 4.3 45.9 М Эй 42.8 9 4.3 44.7 М 3.6 0 10 4.3 43.6 М 3.9 0 11 4.2 43.8 М 3.4 0 12 4.1 41.9 М 2.0 0 13 4.1 43.6 М a) 44.6 14 40 42.9 M ore 16.9
15 3.9 44.4 M 1 0
16 3.9 465.1 M 2.9 0 17 3.9 45.0 М 2.8 0 18 3.9 40.8 М 2.8 0 19 Ban a SM ER 0 20 JON ane M 2:7. 0
21 9.9. 42:0 M 2.6 0
22 CAS AO SA 268 0 23 3.4 40.8 M 2.6 0 24 31 46.2 M 2,8 41.9
25 2.5 0
26 2.4 0
27 2.4 0
28 2.9 42.5
29 2.2 0
30 2.0 0
31 1.9 0

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P. bidens S. paestana

Soi ern Pla Cal C.S.

79 46.4 M 7.1 40.8 M
5.3, (40.2 M 48 44.4 M
44 39.1 M 43 44.9 M
4.2 40.3 M 42 41.0 M
4.0 44.5 M 4.1 39.9 М
99 42.7 М т oe М 3.7. 430 M 3:7. 143.2 M 3.6 44.3 M 36 345. ЭМ 3.5: "BO М 3.6 40.1 M D 450 M dus. ES M
3.4 42.5 M 33.0. AT M
3,4 1% 91:42 SM 3.8 0 ji
33: 63898 M 3.00 148,5 M 3.3 40.7 М 3.2 44.4 М т. 30 М 3.2 41.5 М 3.2 44.5 М 3.2 42.6 М 28 23,1 М 3.2 44.1 М 2.9 42.9 М 31 483,5 M 29 44.8 M 31 791.2 "SM
AT 2 AT M 21 ‘429 M
27.420 M 29 096 M 2.6 43.7 M 2.8 43.0 M
26). 32.5 -8M 2.8 0 hi
24 46.5 M 2.8 0 7
23. 438 M 20 41,6 M 23 42.9 M 22“ 430 M 20 425 M 2.2 44.8 M
2.0 43.0 М 21...45. а М
20. 305 SM 21 427 M
les 422 M 2.0 0 №
16 1408 М Au Gt М

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H. bosniensis (Thaler, 1963) and C. laminata
(Thaler, 1963; Rainer, 1967). The comparison
of karyological data with the phylogentic tree
proposed by Uit de Weerd & Gittenberger
(2013), provides useful information (Fig. 3;
note that the phyletic positions of Medora sp. and S. paestana is on the basis of evidence of phylogenetic tree by Uit de Weerd et al. (2004)). Each of the two main clades includes species both with 2n = 48 and 2n = 62, suggesting that chromosome rearrangements affecting the number of elements occurred independently in the two main clades. However, the karyotypes of the two Delimini, P bidens and S. paestana, differ in showing a diverse number of telocentric elements, none in the former species and four pairs in S. paestana. The differences between the two karyotypes probably originated by four events of inversion or, as recently evidenced in mammals from comparative genome analysis (Stanyon & Bigoni, 2010; Rocchi et al., 2012), of centromere repositioning. In Medorini, in addition to the rearrangements of the chromosome number, also events of inversion (or centromere repositioning) have to be considered. In fact, seven fusions (or fissions) and eight inversions occur for the transition between the karyotypes of Medora sp. (2n = 62; NF = 80) and L. candidescens (2n = 48; NF = 96). Unfortunately, karyologi-cal data are not sufficient to surely assess the polarity of the rearrangements which operated during Alopiinae species diversification. In this context, the results of NORs are unhelpful. Comparable data of NORs in Pulmonata are scarce (see reference in Thiriot-Quiévreux, 2003), while, so far, those ones obtained by FISH with rDNA probes, concern two Helicidae (Vitturi et al., 2005), and four slugs (Vitturi et al., 2004; Colomba et al., 2009). In those FISH evidence, loci of NORs were observed either on a single or multiple chromosome pairs, which are considered, respectively, a primitive and derivate character in several taxa, including molluscs (Thiriot-Quiévreux, 2002, 2003; Wang & Guo, 2004; Colomba et al., 2009; Petraccioli et al., 2012). Then the four investigated Alopii-nae species, possessing NORs on a single chromosome pair, display the plesiomorphic (ancestral) condition.
In other 2n = 62 Pulmonata species, for example, some arionid slug species, the first chromosome pair is not distinctively bigger than the other pairs (Patrao et al., 2013).

Conversely, in Medora sp., S. paestana and P. bidens the chromosomes of the first pair are distinctively larger than ones of the other pairs. The chromosomal similarities of the three Alopiinae species, namely 2n = 62 with the first pair distinctively bigger than the other pairs, are intriguing and candidate as plesiomorphic, because showed by species of either main clades of Alopiinae.
Our chromosomal data show a relevant degree of intergeneric karyotypic diversity in Alopiinae concerning both number and/or shape of chromosomes. Probably, the process of karyological diversification still is ongoing in this group of door snails, as suggested by the presence of gaps on chromosomes of S. paes-tana. Indeed, this door snail species shows high frequency of gaps on the subterminal regions of an achiasmatic medium small bivalent. Chromosome gaps represent hot spots for recombination (Yunis & Soreng, 1984; Glover & Stein, 1988) and their expression can be induced by genomic or environmental stress (Durkin & Glover, 2007). In S. paestana, we consider improbable a possible involvement of environment, because the studied samples came from a pristine areas in the Cilento, Vallo di Diano and Alburni National Park. So, the presence of two fragile sites on a medium small bivalent of S. paestana suggests a pro-pensity of this chromosome pair to structural rearrangements.

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LITERATURE CITED

COLOMBA, M. S., R. VITTURI, M. RAMPIN, A. LANNINO, A. TARAVELLA & A. LIBERTINI, 2009, Comparative cytogenetic analysis of three Stylommatophoran slugs (Mollusca, Pul-monata). Malacologia, 51: 173-179.
COLOMBA, M. S., F. LIBERTO, A. REITANO, W. RENDA, G. POCATERRA, A. GREGORINI & |. SPARACIO, 2012, Molecular studies on the genus Medora H. etÀ . Adams, 1855 from Italy (Gastropoda Pulmonata Clausiliidae). Biodiver-sity Journal, 3: 571-582.
DURKIN, $. С. & T. W. GLOVER, 2007, Chromo-some fragile sites. Annual Review of Genetics, 41: 169-192.
GIUSTI, F., 1967, Notulae Malacologicae 1. Sulla sistematica e distribuzione del genere Medora M. etA . Adams nell’Italia appenninica. Archivio Botanico e Biogeografico Italiano, 43: 406-424.
GIUSTI, F., 1971, Notulae malacologicae XVI. | molluschi terrestri e di acqua dolce viventi sul massiccio dei Monti Reatini (Appennino Centra-le). Lavori della Societa italiana di Biogeografia (N.S.), 2: 423-576.
GIUSTI, F., С. GRAPPELLI, С. MANGANELLI, R. FONDI & L. BULLINI, 1986, An attempt of natural classification of the genus Medora in Italy and Yugoslavia, on the basis of conchological, anatomical and allozymic characters (Pulmo-nata: Clausiliidae). Lavori Società italiana di Malacolologia, 22/1984: 259-362.
GIUSTI, F. & G.MANGANELLI, 1992, The problem of the species in malacology after clear evidence of the limits of morphological systematics. Pp. 153-172, in: E. GITTENBERGER & J. Goup, eds., Proceedings of 9th International Malacological Congress Edinburgh, 31 August-6 September 1986. Unitas Malacologica, Leiden, 414 pp. GLOVER, T. W. & C. K. STEIN, 1988, Chromo-some breakage and recombination at fragile sites. American Journal of Human Genetics, 43: 265-273.
KUSTER, H. C., 1847 [1844-1862], Die Schliessschnecken und die verwandten Gattun-gen (Clausilia, Balea, Cylindrella, Megaspira.), mit Beitragen von Dr. Philippi. In Abbildungen nach der Natur mit Beschreibungen. Systema-tisches Conchylien-Cabinet, 1(14): 355 pp., 38 pls. Nürnberg.
LEVAN, A., К. FREDGA & А. A. SANDBERG, 1964, Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201-220. LINNAEUS, C., 1758, Systema naturae per regna tria naturae ... editio decima, reformata, Vol. 1. Animalia. L. Salvii, Stockholm, 824 + Ш pp. NORDSIECK, H., 2007, Worldwide door snails (Clausiliidae) Recent and fossil. ConchBooks Press, Hackenheim, 214 pp.
NORDSIECK, H., 2009, Ergänzung der Revision der Gattung Medora H. & A. ADAMS, mit Be-schreibung neuer Taxa (Gastropoda, Stylom-matophora, Clausiliidae, Alopiinae). Conchylia, 40: 2-11.

NORDSIECK, H., 2012, Ergänzung der Revision der Gattung Medora Н. & A. ADAMS: Die Medo-ra-Arten Italiens (Gastropoda, Stylommatopho-ra, Clausiliidae, Alopiinae), mit Beschreibung einer neuen Unterart von Medora dalmatina Rossmässler. Conchylia, 42: 75-81. NORDSIECK, H., 2013a, Comment on Uit de Weerd & Gittenberger (2013): Phylogeny of Clausiliidae. http://www.hnords.de/535642a12 5135e10d/535642a211106da02/index.html. NORDSIECK, H., 2013b, Delimini (Gastropoda, Pulmonata, Clausiliidae) from Apennine Italy, with the description of three new subspecies from Calabria. Conchylia, 44: 3-14.
ODIERNA, G., G. APREA, M. BARUCCA, M. A. BISCOTTI, T. CAPRIGLIONE & E. OLMO, 2008, Karyology of the Antarctic chiton Nut-tallochiton mirandus (Thiele, 1906) (Mollusca: Polyplacophora) with some considerations on chromosome evolution in chitons. Chromosome Research, 16: 899-906.
PARK, С. M., 2007, Cytological study of Euphae-dusa fusaniana (Stylommataphora: Clausiliidae) of Korea. Korean Journal of Malacology, 23: 59-63.
PATRÄO, C., J. TEIXEIRA DE SOUSA, K. JOR-DAENS, T. BACKELJAU, R. CASTILHO & A. LEITAO, 2013, Geomalacus and Letourneuxia (Mollusca Pulmonata): a cytogenetic assess-ment. Malacologia, 56: 333-338. PETRACCIOLI, A., N. MAIO & G. ODIER-NA, 2012, Chromosomes of Lepidochitona caprearum (Scacchi, 1836) (Polyplacophora, Acanthochitonina, Tonicellidae) provide insights into Acanthochitonina karyological evolution. Comparative Cytogenetics, 6: 397-407. PHILIPPI, R. A., 1836, Enumeratio molluscorum Siciliae cum viventium tum in tellure Tertiaria fossilium quae in itinere suo observavit, vol. 1. Schroppi, Berolini [Berlin], xiv + 268 pp., 12 pls. RAINER, M., 1967, Cromosomenuntersuchungen an Gastropoden (Stylommatophora). Malacolo-gia, 5: 341-373.
КЕПАМО, А., Е. LIBERTO & I. SPARACIO, 2007, Nuovi dati su Molluschi terrestri e dulciacquicoli di Sicilia. 1° Contributo (Gastropoda Prosobran-chia Neotaenioglossa; Gastropoda Pulmonata Basommatophora, Stylommatophora). // Natu-ralista Siciliano, 31: 311-330.
ROCCHI, M., N. ARCHIDIACONO, W. SCHEMPP, O. CAPOZZI & R. STANYON, 2012, Centro-merere positioning in mammals. Heredity, 108: 59-67.
ROSSMASSLER, E. A., 1835 [1835-1837], Iconographie der Land- und Süsswasser-Mollusken, mit vorzüglicher Berücksichtigung der Europäischen noch nicht abgebildeten Arten, vol. 1: Vi + 132+ 260% 33+ 27 + 70 pp. 30 pls.
STANYON, К. & Е. BIGONI, 2010, Primate chro-mosome evolution: with reference to marker order and Neocentromeres. Russian Journal of Genelics, 46: 1087-1093.
THALER, E., 1963, Beiträge zur Cytologie der Clausiliidae (Gastropoda, Pulmonata. Stlom-

232 PETRACCIOLI ET AL.

matophora). Osterreichische Akademie der Wissenschaften, 24: 1-19.
THIRIOT-QUIEVREUX, C., 2002, Review of the
literature on bivalve cytogenetics in the last ten
years. Cahiers de Biologie Marine, 43: 17-26. THIRIOT-QUIEVREUX, C., 2003, Advances in chromosomal studies of gastropod molluscs. Journal of Molluscan Studies, 69: 187-201.
UIT DE WEERD, О. R., W. H. PIEL 8 E. GITTEN-BERGER, 2004, Widespread polyphyly among Alopiinae snail genera: when phylogeny mirrors biogeography more closely than morphology. Molecular Phylogenetics and Evolution, 33: 533-548.
UIT DE WEERD, D. К. 8 Е. GITTENBERGER, 2013, Phylogeny of the land snail family Clau-siliidae (Gastropoda: Pulmonata). Molecular Phylogenetics and Evolution, 67: 201-216. VITTURI, R., L. SINEO, N. VOLPE, A. LANNINO & M. S. COLOMBA, 2004, Repetitive DNAs in

the slug Milax nigricans: association of ribo-somal (18S-28S and 5S rDNA) and (TTAGGG) ntelomeric sequences) in the slug M. nigricans (Mollusca: Gastropoda: Pulmonata). Micron, 35: 255-260.
VITTURI, R., A. LIBERTINI, L. SINEO, I. PARA-CIO, A. LANNINO, A. GREGORINI & М. $. COLOMBA, 2005, Cytogenetics of the land snails Cantareus aspersus and С. mazzullii (Mollusca: Gastropoda: Pulmonata). Micron, 36: 351-357.
WANG, Y. & X. GUO, 2004, Chromosomal rear-rangement in Pectinidae revealed by rRNA loci and implications for bivalve evolution. Biological Bulletin, 207: 247-256.
YUNIS, J. J. & A. L. SORENG, 1984, Constitu-tive fragile sites and cancer. Science, 226: 1199-1204.

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